PHYLOGENETIC PLACEMENT OF A RECENTLY DESCRIBED TAXON OF THE GENUS *PLEODENDRON* (CANELLACEAE)

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ABSTRACT

In order to determine placement of the recently described plant species, *Pleodendron costaricense* (Canellaceae), five DNA regions were sequenced. For the new species, those included two from the nuclear rRNA coding region, ITS and 18S, and three from the chloroplast genome, the genes for *rbc*L and *atp*B and the spacer trnLF region. For the 18 taxa of Canellaceae and sister group Winteraceae, ITS and *trn*LF sequences were published (Karol et al. 2000), while the other three regions were sequenced for this study. The aligned sequences were combined and analyzed with parsimony, likelihood and Bayesian programs. The single tree produced in these analyses provided 100% support for placement of *P. costaricense* in a monophyletic group with *Pleodendron macranthum* and *Cinnamodendron ekmanii*. This result suggests that nomenclatural changes for those three species should be considered. *Phytologia 94(3): 404-412 (December 1, 2012).*

KEYWORDS: Canellaceae, *Pleodendron*, nuclear ribosomal DNA (nrDNA), chloroplast DNA (cpDNA), phylogenetic analyses

The family Canellaceae, along with the family Winteraceae, make up the order Canellales (Stevens 2010). Canellaceae is a relatively small family (~16-20 species) of woody plants. Like the Winteraceae, the Canellaceae have a disjunct distribution, with species in the New World (South America and the Caribbean) and Africa, including Madagascar (Kubitzki 1993). Although several papers have concentrated on molecular phylogenetic relationships of the Winteraceae (Suh et al. 1993; Karol et al. 2000; Doust and Drinnan 2004; Marquinez et al. 2009) using nuclear ribosomal and chloroplast DNA spacers, those investigations used one to six species of Canellaceae, each from a different genus, and primarily as outgroups. Recently, a new species of Canellaceae was discovered in Costa Rica and described as *Pleodendron costaricense* (Hammell and Zemora 2005). We wished to place this new species in the molecular phylogeny produced in our previous paper that highlighted Winteraceae. To accomplish this, we sequenced *P. costaricense* for the ribosomal DNA (nrDNA) spacer ITS and the chloroplast DNA (cpDNA) region *trn*LF, as in Karol et al. (2000). We also increased the number of phylogenetically informative characters for all 19 taxa, adding sequences for their chloroplast genes *rbc*L and *atp*B and for their 18S nuclear ribosomal region. The single tree produced in our analyses differs from that found in a recent article of Salazar and Nixon (2008).

MATERIALS AND METHODS

Species sampled and sources of plant material: With the exception of the new accession of *P. costaricense*, vouchered as *Zamora et al. 2986* (Hammell and Zamora 2005), all samples and voucher numbers were those listed in Table 1 of Karol et al. (2000).

DNA amplifications and sequencing: DNA isolation and subsequent amplification and sequencing for the nrDNA ITS and *trn*LF regions were conducted for the *Pleodendron costaricense* sample as described in Karol et al. (2000). The 18S rDNA, *rbc*L and *atp*B regions were amplified from the 18 DNA samples obtained for the Karol et al. (2000) study along with the new *Pleodendron costaricense* sample. Primers and amplification conditions for all 19 taxa used were those from Karol et al. (2000) and Soltis et al. (2000). Sequencing was

performed according to standard procedures on an ABI automated sequencing instrument. Alignments were produced with the program Se-Al vsn 2.0a11 Rambaut (2000). ITS and *trn*LF alignments, which include a significant number of insertion/deletion characters, were added with the alignment of Karol et al. (2000) as a guide. The 18S region, because of its slow rate of change, and the *atp*B and *rbc*L sequences that encode proteins, these sequences were straightforward and aligned by eye.

Phylogenetic analyses: Analyses were performed for each single gene, for the two nuclear sequences combined, for the three chloroplast sequences combined and for the complete concatenated data set. Parsimony analyses were performed in PAUP* (Swofford, 2002) using the Branch-and-Bound algorithm and default parameters. Winteraceae sequences were used as the outgroup for the Canellaceae. Parsimony bootstrapping was done for 1000 replicates, again with branch-and-bound settings. Maximum likelihood analyses on the concatenated and individual data sets were performed with PAUP*, on a 32 processor cluster, using parameters settings derived from three iterations of ModelTest (Posada and Crandall, 1998). GARLI (Zwickl, 2006) gave a similar tree. Bayesian analyses were performed on the data sets with MrBayes (Huelsenbeck and Ronquist, 2003), also on the computer cluster. ILD and SH tests (Farris et al.,1994; Kishino and Hasegawa, 1989) were performed to determine whether the data from all five DNA regions could be combined.

RESULTS AND DISCUSSION

As noted above, alignment of the new sequences were easily accomplished by eye. In the concatenated data set (available upon request), positions 1-1411 represent *rbc*L; 1412-2855 *atp*B; 2856-4568 18S; 4569-5555 *trn*LF; and 5556-6334 ITS. For the new 19 taxa data sets (i.e., for *rbc*L, *atp*B and 18S) only minor indels were identified. The first, in *rbc*L, is a three-codon difference just before the UUA stop codon; the Winteraceae have a GAU (Asp) GTC (Val) UUG (Leu) sequence, whereas the Canellaceae is missing those three codons. The second, an additional AAU (Asn) codon in position 34 of *atp*B, is also present in *Cinnamodendron ekmannii* and the two *Pleodendron* species, but absent in all other taxa. Of the seven single base differences in the

18S rDNA sequence, six are autapomorphies in Winteraceae, and the seventh is informative in Winteraceae (C) and Canellaceae (T) overall, but autapomorphic in *Takhtajania* (-) and in *Pleodendron costarciense* (A). No new indels, relative to those in Karol et al. (2000), were identified for *trn*LF with the inclusion of the new *P. costaricense* sequence. For ITS, the region with the most indels in the published 18 taxon data set, only two autapomorphies, both single base insertions in *P. costaricense* relative to the other species, were observed.

The five individual DNA regions, the combined three chloroplast DNA regions, and the combined two rDNA regions were first analyzed separately to check for incongruence. The results of these ILD and SH tests indicated that combining the data sets did not violate the null hypothesis. With unweighted maximum parsimony, using the Branch-and-Bound option in PAUP*, a single most parsimonious tree was obtained for the concatenated data set, as well as for ITS separately (Fig.1). For *trn*LF alone, two trees with the same topology as in Karol et al. (2000), were obtained. Individual 18S, *rbc*L and *atp*B DNA trees were more unresolved, with the 18S region exhibiting the most polytomies. Combining the nuclear rDNA regions also gave a single tree identical to the ITS tree. Combining the chloroplast DNA regions resulted in 36 most parsimonious trees whose consensus generally agreed with the nrDNA trees, but was also less well-resolved. Bootstrap support values based on 1000 replicates for the combined sequence tree are given (Fig.1). Likelihood and Bayesian analyses gave the same topology as did maximum parsimony. The bootstrap values for likelihood, and the posterior probabilities for the latter, are also given on the tree (Fig. 1). In the single tree produced, and with all three algorithms, *Pleodendron costaricense* was in a clade with *Pleodendron macranthum* and *Cinnamodendron ekmannii*, with 100% support values. However, that clade had *Pleodendron* as a paraphyletic lineage, with *P. macranthum* and *C. ekmanii* actually forming a clade with 100% support values, and *P. costaricense* basal to that clade. Relationships of the four other Canellaceae were those seen in the study of Karol et al. (2000), albeit with much stronger support for the monophyly and the exact branching relationships of *Warburgia salutaris*, *Cinnamosma madagascariensis* and *Capsicodendron denisii*. *Canella winterana* remained outside that clade, but with no support as to its ultimate affinity.

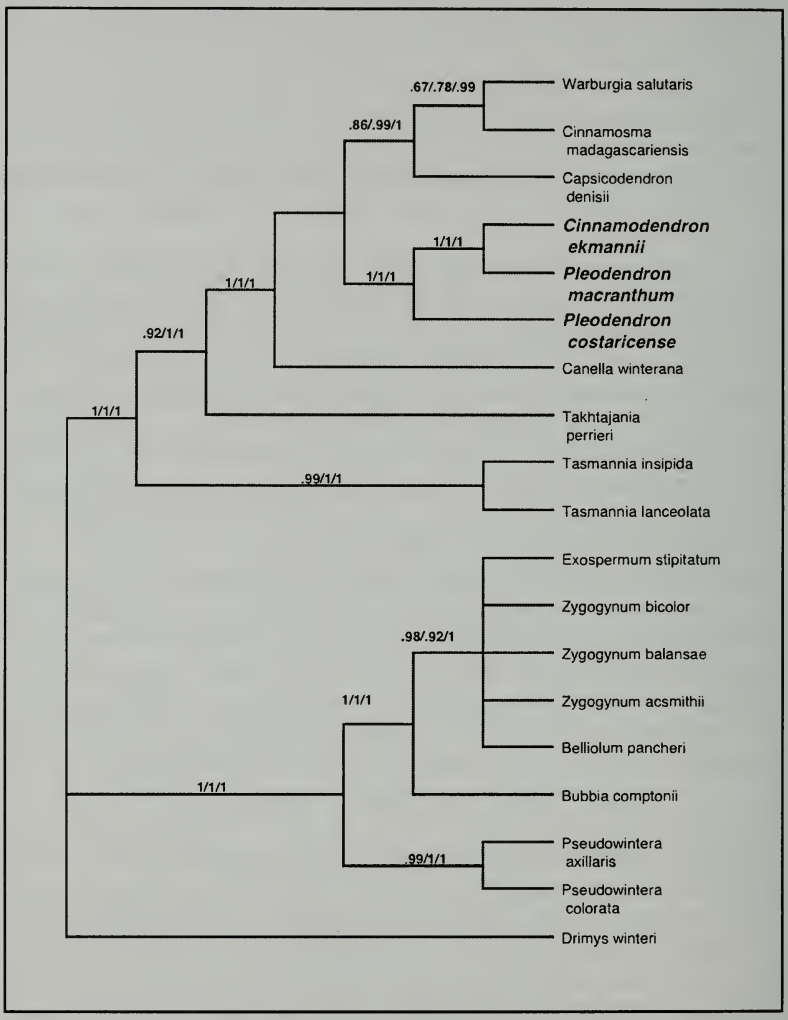

Figure 1. Most parsimonious single tree for Canellales based on combined data from five gene regions. Numbers above the nodes, from left to right, are for: maximum parsimony bootstrap support; maximum likelihood bootstrap support; Bayesian posterior probabilities.

The Winteraceae relationships, including *Takhtajania* as the basal lineage, and the polytomy seen for the closely related *Zygogynum/Exospermum/Belliolum* clade were basically those found in our previous study (Karol et al. 2000).

With the addition of data from three additional coding sequences, we continued to generate a phylogeny for the Canellales that agreed with our previous one in Karol et al. (2000). The placement of the new taxon described as *Pleodendron costaricense* was unequivocally shown to be sister to *Cinnamodendron ekmannii* and *Pleodendron macranthum* (Fig. 1). Given our results with the three taxa forming a completely supported monophyletic clade, these taxa should be combined into a single genus, presumably as *Cinnamodendron*, which has taxonomic priority (Kubitzki 1993).

Our study is in conflict with that of Salazar and Nixon (2008) for 49 morphological characters, and molecular data sets for five markers, three of which, ITS, *trn*LF and *rbc*L, are in common with ours. They also used the chloroplast *mat*K gene and the spacer *trn*D-*trn*T, whereas we included 18S rDNA and *atp*B. Their study included *Pleodendron costaricense* as well as several additional Antillean and South American species denoted as members of the genus *Cinnamodendron*. In addition to using Winteraceae as an outgroup, they also included four more distantly related "magnoliid" genera, *Illicium*, *Annona*, *Myristicum* and *Piper*. Unlike our results, Salazar and Nixon's phylogeny placed the two *Pleodendron* species as well-supported sister species which were in a monophyletic clade with the Antillean *Cinnamodendron*. Their consensus tree (of 42 most parsimonious ones) also placed *Capsicodendron* with the South American *Cinnamodendron*, unlike our single tree with *Capsicodendron* strictly aligned with *Warburgia* and *Cinnamosma*.

The discrepancy between the two studies is somewhat difficult to resolve. The differences for two markers are likely to cause this, as the topology of our tree is observed for ITS, *trn*LF and *rbc*L alone, with ITS providing the majority of base substitution and indel characters. At least some of the differences observed for our findings, versus those for the Salazar and Nixon, paper may be due to alignment issues for ITS and the chloroplast spacers. Unfortunately, their paper does not include

a Materials and Methods section describing their alignment and analyses procedures. Additionally, for the four outgroup magnoliids, we have not found it possible to align their ITS sequences with those of the Canellales (Suh et al. 1993). When their additional ITS, *trn*LF and *rbc*L sequences available in GenBank were included in our aligned data set for maximum parsimony and bootstrap analyses, we generated the same phylogeny as that in Fig.1 with respect to the *Pleodendron costaricense-Pleodendron macranthum-Cinnamodendron ekmanii* clade and the *Capsicodendron dinesii-Warburgia salutaris-Cinnamosma madagascariensis* one (data not shown). We did find that the Antillean *Cinnamodendron* species were allied with the currently named *Pleodendron* species, separate from the South American ones (which were allied with the *Capsicodendron dinesii-Warburgia salutaris-Cinnamosma madagascarensis* clade), as did Salazar and Nixon, so it is likely that their recommendation for naming a new genus for the South American group is appropriate.

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